IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	:	09/991,971	Confirmation No. 8814
Applicant	:	Markku AHOTUPA	
Filed	:	26 November 2001	
TC/A.U.	:	1644	
Examiner	:	Phuong N. Huynh	
Docket No.	:	2630-113	
Customer No.	:	6449	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.131(a)

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

We, Markku AHOTUPA, John ERIKSSON, Lauri KANGAS, Mikko UNKILA, Janne KOMI, Merja PERÄLÄ, and Helena KORTE, applicants for the above-identified patent application, declare as follows:

1. That some time on or prior to 21 March 2001, the inhibition of overactivity of phagocytes by administering hydroxymatairesinol identified in the instant application had been determined. That is, a human neutrophil sample was stimulated by addition of phorbol-myristate-acetate (PMS) to produce an oxidative burst. Addition of hydroxymatairesinol to the human neutrophil sample was found to inhibit oxidative burst stimulated by PMA.

2. That some time on or prior to 21 March 2001, the inhibition of overactivity of phagocytes by administering hydroxymatairesinol identified in the instant application had been determined. That is, a porcine neutrophil sample treated with hydroxymatairesinol was found to inhibit myeloperoxidase activity.

3. All of the above experiments were performed in laboratories at MCA Research Laboratory Ltd in Turku, Finland.

4. The date of the determination for each inhibition of overactivity was determined from notebook records. Copies of the notebook records evidencing the determination of the above inhibition of overactivities are attached hereto as follows:

Exhibit 1

Primary finding on inhibition of human neutrophil oxidative burst by hydroxymatairesinol was done in laboratories at MCA Research Laboratory Ltd. The assay was performed under our direction and supervision by research associate, Ms. Riikka Hirsinummi (RH). For measurement of oxidative burst she used Bio Orbit 1251 Luminometer (documents 1.1 and 1.2: copies of equipment notebook). Copies of of notes on her notebook (documents 1.3 and 1.4) show dilutions used and an outline of graphical presentation of reults. A copy of original data sheet is also enclosed (document 1.5).

Exhibit 2

Primary finding on inhibition of porcine neutrophil myeloperoxidase activity by hydroxymatairesinol was done in laboratories at MCA Research Laboratory Ltd. The assay was performed under our direction and supervision by research associate, Ms. Riikka Hirsinummi (RH). For measurement of myeloperoxidase activity she used Perkin Elmer UV/VIS Spectrophotometer Lambda 2 (documents 2.1., 2.2 and 2.3: copies of equipment notebook). Copy of her notes on her notebook (document 2.4) shows dilutions used. A copy of original data sheet is also enclosed (document 2.5).

These notebook records indicate that the above inhibition of overactivities were discovered prior to the corresponding dates set forth in the above paragraphs 1-2. All dates have been redacted in the attached photocopy of the relevant laboratory notebook pages so as to maintain the confidentiality of the actual date of invention.

5. It is further declared that the accompanying exhibits may not be a complete record of applicants' data concerning the invention of the instant patent application and are not necessarily meant to represent the earliest date of conception. The accompanying exhibits are presented

solely to prove a completion of the invention prior to the date of the Yesilada et al. (03/21/01) prior art cited by the Examiner in the Office Action dated 21 October 2004.

The declarants further state that the above statements were made with the knowledge that willful false statements and the like are punishable by fine and/or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that any such willful false statement may jeopardize the validity of this application or any patent resulting therefrom.

Dated: Turku Feb 14, 2005

Markku AHOTUPA

Dated: Turku Feb 18, 2005

John ERIKSSON

Dated: Turku Feb 15, 2005

Lauri KANGAS

Dated: Turku Feb 15, 2005

Mikko UNKILA

Dated: Turku Feb 15, 2005

Janne KOMI

Dated: Turku Feb 15, 2005

Merja PERÄLÄ

Dated: Turku Feb 16 2005

Helena KORTE

document 1.1 equipment notebook

Bio Orbit
1251 LUMINOMETER

'Vanha'

document 1.2 equipment notebook

					SIGNAL
LS	VASANKARI / TYTOT	+37°C	TRAP		0,694-0,703
LS	—"—	+HORMOS	—"—	"	0,694-0,685
LS	—"—	+HORMOS	"	—	0,697-0,703
LS	(196) TYTOT				
LS	HERMOS				
LS	"				
"	"	35°C	SOD		
LS	HORMOS	35°C	SOD		0,861-0,893
LS	PILOTTI	+37°C	TRAP		0,693-0,716
LS	"	"	"		0,707-0,694
LS	"	"	"		0,702-0,697
LS	"	"	"		0,707-0,689
LS	"	"	"		0,715-0,719
LS	HORMOS	33°C	PEROX		0,733-0,741
LS	—"—	—"—	—"—		1,013-1,022
LS	"	"	"		0,716-0,730
LS	"	"	"		0,716+0,721
LS	DANISCO	33°C	PEROX		0,732-0,741
LS	"	"	"		0,724-0,734
LS	DANISCO	35°C	SOD		0,789-0,766
LS	HORMOS	"	"		0,849-0,820
LS	"	"	"		0,750-0,746
LS	HORMOS	37°C	TRAP		
LS	"	"	"		0,754-0,722
RH	OXIDATIVE BURST		OMA RIIKKA OX		
LS	RAMO	35°C	SOD		
LS	"	"	"		0,764-0,757
LS	HORMOS	33°C	PEROX		
RH	OXIDATIVE BURST		RIIKKA OX + SOD		
RH	~~~~		~~~~		
LS	HORMOS	33°C	PEROX		0,702-0,733
LS	HORMOS	35°C	SOD		0,725-0,733
LS	HORMOS	35°C	SOD		0,664, 0,683
LS	HORMOS	33°C	PEROX		
LS	HORMOS	33°C	PEROX		0,998-0,987
LS	HORMOS	35°C	SOD		0,678-0,687
LS	RAMO	35°C	SOD		

ABEL – oxidative burst

document 1.3 **notebook of RH**

Nitecapone 40mg/mL $\frac{40}{1000} = \frac{3,6}{x} \Rightarrow$ 90 µl 40 µl DMSO + 50 µl EtOH

Y-OH-Toremifen 40mg/mL $\frac{5,0}{0,04} \Rightarrow$ 125 µl 100 µl DMSO + 25 µl EtOH

HW Nitec. 265,2 g/mol

Y-OH-Tor

Jutkitnan eriset HM-laimennokset + Nitecapone + Y-OH-Toremifen

450 µl Reconst-puskuria kyvettiin

+ 100 µl huoneenna vettä laimennos vedestä 10 µl vettä + 1000 µl Lucanis Buffera

+ 100 µl Adjuvant P-mix

+ 10 µl inkubointia aina tähän väliin

SEKOITUS

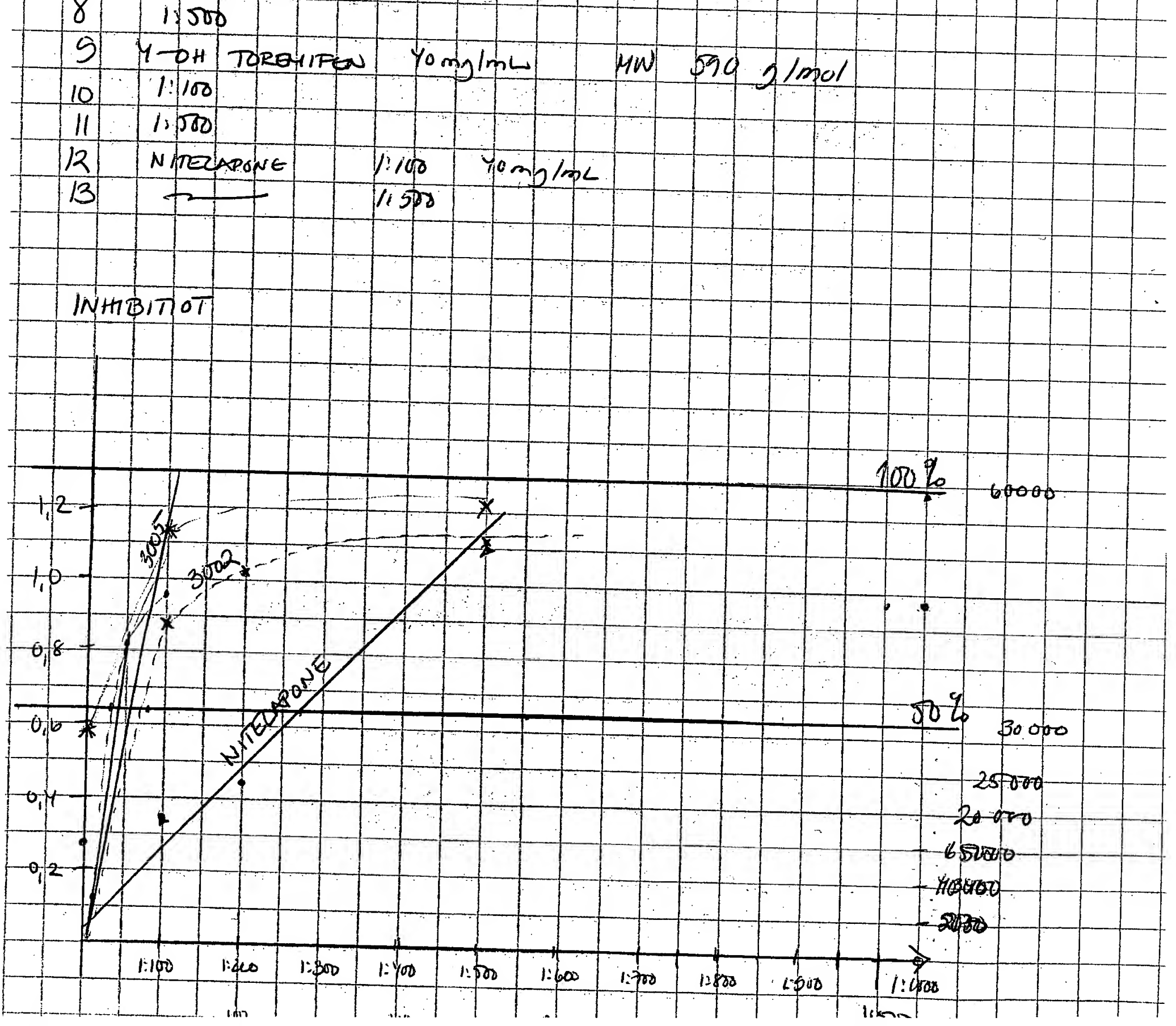
- kyvettiä 37°C ja inkubointi 1 min
- mixaus
- lisätään 250 µl Pholasin:ia
- käynnistetään ajo mittaus 1 min
- injektoinnista TPA
- 100 µl 8 µM + TPA:ta

VSOd ajo 1 HM-3000 5µl 2 eriä 0 5µl EtOH

ajo 2	1	3003 kontrolli	1	kontrolli
	2	1:100	2	1:50
	3	1:200	3	1:100
	4	1:500	4	1:200
	5	3005 kontrolli	5	1:300
	6	1:100	6	1:400

SARJA

1	HM-3005	40 mg/mL	MW 358,4 g/mol
2	1:10		
3	1:50		
4	1:100		
5	HM-3002	40 mg/mL	MW 356,4 g/mol
6	1:100		
7	1:200		
8	1:500		
9	4-OH TORBHIFEN	40mg/mL	MW 590 g/mol
10	1:100		
11	1:500		
12	NITECAPONE	1:100	40 mg/mL
13	—	1:500	



document 1.5 original data sheet

MultiUse 2.0, 1251 Luminometer control program (c) BioOrbit Oy 1993
C:\MULTIUSE\ DAT 04:41:20 Page 1

.01031601.DAT
04:41:20

Sample		Id	time	piikki	tulos
1	0-näyte 5µl EtOH	1	00:00	225.400	62331.400
		2	+00:09	211.500	58721.874
		mean		**218.450**	**60526.637**
		%cv		3.182	2.982
2 (10 mg/mL 5µl)	HH-3000 kaneta 1:50	3	+00:18	27.210	8568.050
		4	+00:27	90.200	28227.825
		mean		~~**58.705**~~	~~**18397.937**~~
		%cv		~~53.650~~	~~53.429~~
3	1:100 1:200	5	+00:36	104.700	33143.600
		6	+00:45	125.400	40161.725
		mean		~~**115.050**~~	~~**36652.662**~~
		%cv		~~9.996~~	~~9.574~~
4	1:300 1:400	7	+00:54	130.100	42285.450
		8	+01:03	138.400	44996.276
		mean		~~**134.250**~~	~~**43640.863**~~
		%cv		~~3.091~~	~~3.106~~

document 2.1 equipment notebook

PERKIN ELMER
UV / VIS
Spectrophotometer
Lambda 2

PERKIN-ELMER
UV/VIS
Spectrophotometer
Lambda 2

F8

OHJELMAT

		LÄHTIEN:	
dc =	Dieenikonjugaatio	SKANNAUS	233-300 nm
dieeni =	Dieenikonjugaatio	mittaus	233 ja 300 nm
mye =	Myeloperoks.daas.		655 nm
kol =	Kolesteroli		500 nm
tba =	tba-määritys		535 nm
gsh =	gsh-määritys		412 nm
folin =	Folin proteiinimääritys		500 nm
dna =	DNA puhtaus ja pitoisuus		260 ja 280 nm
d2nph =	D_2NPH-mittaus		280, 360, 390 nm
lipidper =	Lipidi hydroperoksidi		560 nm

OHJELMAT

db	= Dieeni konjugaatio	233 - 300 nm
mye	= myelopenoksidaasi	655 nm
kol	= kolesteroli	500 nm
tba	= tba-määritys	535 nm.
gsh		412 nm
folin	= 500 nm	

DNA puhtaus 286 ja 260 nm

D_2NPH = 280 nm 360 nm 390 nm

document 2.3 equipment notebook

RH	GXLDL - kokerlijn		DC
LS	PILOTT		DC + KOL
RH	213 HORMOS		KOL + DC
RH	213		HPL2
RH	213		PL2
RH			
RH			
RH	216 HORMOS		
RH			
LS	HORMOS		DC
RH	216 HORMOS		DC + KOL + HPL2
RH			DC + KOL
RH			HPL2
LS	HORMOS		DC
LS	HORMOS		DC
LS	RIM04		GSM
LS	HORMOS		DC
LS	RIM04		FOLIJ
LS	RIM04		MYE
LS	RIM04		FOLIJ
RH	HMR		MYELOPEROXIDASE
LS	HORMOS		DC
RH	HMR		MYE
LS	HORMOS		DC
LS	PILOTT / LYMFOCYTEN		DC + KOL
LS	HORMOS		DC
LS	PILOTT		DC - KOL
RH	HMR		MYE
LS	PILOTT		DC, KOL
LS	PILOTT		DC, KOL
LS	PILOTT		DC, KOL
LS	— " —		— " —
LS	HORMOS		DC
LS	HORMOS		DC
LS	HORMOS		DC
LS	— " —		MYEDOP
LS	222		DC, KOL
LS	— " —		— " —
LS	— " —		— " —

document 2.4 notebook of RH

HM-3000 Myeloperoksidaasi aktiivisuuden määrittäminen

HM-3000 kantaliuos 40 mg/1 ml

20 µl näytettä

HM-3000 Laimennokset

1	kantaliuos	40 mg/mL
2	1:10	
3	1:100	
4	1:500	
5	1:1000	
6	1:2000	
7	1:4000	
8	1:8000	
9	ei HMR ollenkaan	
10		

Catalase

10 mg/mL

500 µg/5mL

0,5 mg/5mL

0,1 mg/1mL

100 : 10 000

document 2.5 original data sheet

UVAVPROG

time: 11:08:38 | method: mye | sample ID: ls000018

	cycle		655.0	
HM-3005 Yomg/mL 1:10	1	1	0.0087	
		1	0.0035	0,0061
		1	0.1181	
		1	0.1251	0,1216
1:50		1	0.8196	
		1	0.8148	0,8172
1:10		2	0.9341	
		2	0.9766	0,9554
HM-3002 Yomg/mL 1:100		2	0.0041	
		2	0.0063	0,0052
		2	0.8654	
		2	0.8786	0,8720

UVAVPROG

time: 11:08:38 | method: mye | sample ID: ls000018

	cycle		655.0	
1:200		3	1.0219	
		3	1.0288	1,025
1:500		3	1.1188	
		3	1.1198	1.1153
Y-OH-tamoxifen Yomg/mL 1:100		3	0.5461	
		3	0.6126	0,5794
		4	1.1344	
		4	1.1421	1,1383
1:500		4	1.2354	
		4	1.1798	1.2076
~~Antagonist~~ Yo	②	4	1.3482	
		4	1.2362	1,2922